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Sex estimation in a contemporary Turkish population based on CT scans of the calcaneus

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Abstract

Building a reliable biological profile from decomposed remains depends heavily on the accurate estimation of sex. A variety of methods based on every single skeletal element have been developed over the years for different populations employing both osteological and virtual methods. The latter seem to be a reasonable alternative in countries lacking osteological reference collections. The current study used 3D virtual models of calcanei from CT scans of living adults to develop a sex estimation method for contemporary Turkish. Four hundred and twenty eight calcanei CT scans were analysed. The sample was divided in two subsamples: an original (N=348) and a validation sample (N=80) with similar distribution of males and females. Nine classical measurements were taken using the 3D models of the calcanei and two different statistical methods (Discriminant function analysis and Binary logistic regression) were used. Classification accuracy ranged from 82% to 98% for the validation sample and it was consistently high using any of the two methods. Sex bias seems to be lower for most of the logistic regression equations compared to the discriminant functions. These results, however, need further testing to be verified. Based on the results of this study we recommend the use of both methods for sex estimation from the measurements of the calcaneus bone in a Turkish population.

Keywords: Forensic Anthropology Population Data, sex estimation, calcaneus, Turkey, Computed Tomography

Introduction

Forensic anthropology has long acknowledged the emerging need for population-specific methodologies in the reconstruction of the biological profile from unknown skeletal remains [1,2]. The assemblage of contemporary skeletal collections over the last decades [3,4] and the contribution of medical imaging and 3D technology, such as 3D reconstruction of bones from Computed Tomography or surface scans (white light, laser), made it possible to acquire biological standards from any population group worldwide.

Sex estimation methods in particular reflect the quantification of shape and size differences between males and females for any given skeletal element [1,2]. The pelvis differs in shape due to the need of the female structure to accommodate childbirth [5] while the other bones of the skeleton are simply smaller in females. These differences are used to develop sex estimation methods based on linear measurements [3,6,7], landmark configurations [8–10] or morphological traits [11]. Amongst these, several studies have focused on foot bones using classical osteometric [7,12–14] and digital techniques [15].

This work focuses on the use of metric characteristics of the calcaneus in sex estimation based on a contemporary sample from Turkey. The calcaneus was selected for this study as it is often well preserved due to the simple fact that modern humans wear shoes and socks especially in cold environments, which protect the foot bones from postmortem alterations and scavenging [16]. The calcaneus plays an important role in locomotion and weight transmission and serves as an indicator of body size in modern humans and other hominids [17,18]. Morphological variation of this bone has been noted since the 1930s with the work of Wells [19] on the differences between Bantu, Bushman and European calcanei. Bunning and Barnett [20] also reported variation on the number of talar articular facets between four different groups (British, Indians, Nigerians and indigenous people from Sri-Lanka). Later studies have demonstrated the utility of this bone in sex and ancestry estimation for various populations [12, 16, 21–26].

Turkey is a large country that houses many different cultures and is located on the border of Eurasia and the Mediterranean coast. The Turkish Human Rights Association (https://en.ihd.org.tr) has so far located numerous mass graves, with over 4000 unidentified individuals [27]. In addition, hundreds of missing persons from the recent 1976 conflict in Cyprus are of Turkish origin, which creates an emerging need for biological standards for this area of the world [28]. The lack of contemporary osteological collections in Turkey has been an obstacle for the development of osteological research in the past. The development of medical imaging technologies in recent years has allowed scholars to develop biological standards from patient's data, and from this data a substantial number of virtual studies have recently emerged [28–32]. This study aims to complement the growing database on osteometric standards for Turkish with the investigation of sexual dimorphism on the calcaneus using 3D reconstructions from CT scans.

Material and methods

In this retrospective study, patients whose CT angiographies were assessed in the Bakırköy Dr. Sadi Konuk Teaching and Research Hospital between June 2014 and January 2015 were investigated. All medical records and CT images of patients admitted to the different clinics of the hospital were evaluated. Cases that had fracture, surgery, deformity or showed pathological conditions in the calcaneus were excluded from the study (5 cases). Finally, 408 patients with an age range of 18-99 years were included (218 males, mean age: 38.21±15.9 and 210 females, mean age: 37.02±16.6 and overall mean age for both sexes was 34.44±16.4). Only the left calcaneus was measured. Multi-detector CT (MDCT) examinations were performed using a 128-slice MDCT scanner (Siemens Medical Solutions, Erlangen, Germany).

All scans were obtained with the patients laying supine using the following parameters: tube voltage, 120 kV; effective mAs, 150; slice thickness 1 mm. MDCT images were obtained using 3D reconstructions and a volume-rendering technique (VRT). Image processing then starts with the manual separation of the calcaneus from other skeletal parts. Separation is a process that allows the virtual separation of regions with different density in the image. In this case, the authors used a manual segmentation method.

All images were measured at the Leonardo workstation (Siemens, Germany, running Siemens syngo® MMWPVE52A software). In total, nine measurements were marked on the left side of the 3D reconstructed calcaneus. Nine measurements were collected from each calcaneus as described by Martin and Saller [33] and Bidmos [26] (Figure 1). A description of each measurement can be found below:

Maximum length (MAXL): Linear distance between the most anterior point of the calcaneus and the most posterior point on the calcaneal tuberosity.

Load arm length (LAL): The projected distance between the most anterior point on the calcaneus and the most posterior point on the posterior articular facet (5a following Martin and Saller [33]).

Minimum breadth (MINB): Minimum distance between the medial and lateral surfaces of the body of the calcaneus.

Middle breadth (MIDB): Linear distance between the most lateral point on the posterior articular facet and the most medial point on the sustentaculum tali.

Body height (BH): Linear distance between the superior and the inferior surfaces of the body of the calcaneus taken on the coronal plane, at the midpoint between the most posterior point of the posterior articular facet and the most anterior point of the calcaneal tuberosity.

Maximum height (MAXH): Distance between the most superior and the most inferior points on the calcaneal tuberosity.

Dorsal articular facet length (DAFL): Linear distance between the most posterior and the most anterior points on the posterior articular facet of the calcaneus.

Dorsal articular facet breadth (DAFB): Linear distance between the most medial to the most lateral points on the posterior articular facet.

Cuboidal facet height (CFH): Linear distance between the most superior and the most inferior points on the cuboidal articular facet.

Reliable replication of the measurements is an essential component of any metric method. In this study, all of the images were assessed by two experienced radiologists (over 10 years) in musculoskeletal assessments. Two weeks after the first radiology assessment of the CT images, both radiologists reassessed 40 randomly selected 3D models. Intra- and inter-observer error were performed using Technical Measurement Error (TEM), relative TEM (rTEM) and the coefficient of reliability (R) of the measurement as suggested by Ulijaszek and Kerr [34].

The data was divided in two groups: the original group (O) consisting of 348 individuals (179 males and 169 females) and the validation group (V) consisting of 80 individuals (39 males and 41 females). Group V was randomly selected from the total sample in order to avoid any selection bias. Group O was used to generate formulae to discriminate sexes while Group V was used for cross-validation.

Mean differences of the variables between males and females for Group O were tested using an independent t-test.

The statistical analysis of Group O was done using two common discriminant methods:

a) Discriminant Function Analysis (DFA), which is a statistical method that assumes certain conditions for the distribution of the data (normality, equality of the covariance matrices). Univariate and multivariate discriminant functions (DF) were developed for different combinations of variables in direct and stepwise analyses. Stepwise discriminant function analysis with a Wilks' lambda criterion and using an alpha of 0.05 was performed. Wilks' lambda test is to test which variables contribute significance in a discriminant function. The closer Wilks' lambda is to 0, the more the variable contributes to the discriminant function. Conversely, the bigger the eigenvalue, the stronger is the discriminating power of the function. The stepwise discriminant functions were produced for all the measurements taken on the calcaneus. Normality of the sample was tested using the Kolmogorov-Smirnov test.
b) Binary logistic regression was also performed in the same dataset. This method is adequate for non-parametric data; meaning it is not bound by any assumptions on the data distribution. The same procedure was followed as with DFA and univariate and multivariate functions (LOGR) were developed in direct and stepwise analyses.

Statistical Package for the Social Sciences (Version 17, SPSS Inc., Chicago, IL) software was used for the statistical analyses. Statistical significance was set at $p<0.05$.

Results

The Kolmogorov-Smirnov test indicated a normal distribution of the sample ($p > 0.05$). The covariance matrices were not equal in some occasions.

Inter- and intra-observer error were calculated using TEM, rTEM and R as seen in Table 1. The rTEM was <5% in all cases and R was >0.9 with the exception of DAFB and DAFL. In general, observer agreement is fair and consistent with other studies.

Descriptive statistics (mean, standard deviation, and p-values) for the calcaneus are presented in Table 2. As expected, all the measurements of the calcaneus were significantly larger in males than in females ($p < 0.001$), suggesting that all of these measurements could be useful as sex indicators. The difference between the two groups is much more evident for MAXL (about 10 mm difference between the two means). These results demonstrate the presence of marked sexual dimorphism in the calcaneus bone of this Turkish sample, and thus metric analysis of these skeletal elements should provide an effective method for the estimation of sex in this population group.

Univariate Discriminant Functions and Logistic Regressions

Univariate discriminant function analysis was carried out for all single variables and the demarking point was calculated (see Table 3). The single variable with the highest classification accuracy was MINB, which showed 100% correct classification for the original sample and 90% classification accuracy for the validation sample. Sex bias for the validation sample was estimated to be 19.5% in favour of the male group. The lowest sex bias for the validation sample was noted for MAXL (-0.5%) and for DAFB (-7.9%). In both cases females gave higher classification accuracies compared to males.

Logistic regression equations were also developed for each single variable and the results are summarised in Table 3. The MINB also gave the highest overall classification accuracy (100%) for the original data. Yet, classification accuracy for the validation sample barely reached 89% with a sex bias of 22%. The lowest sex bias was noted for MIDB (1.9%) and DAFB (-5.4%).

Both methods gave similar results for the original and validation samples in all cases. Logistic regressions gave lower sex bias in all cases for the original sample and in 5/8 cases for the validation sample. The DAFB is the best single variable combining high overall classification accuracy and low sex bias for the validation sample.

Multivariate Discriminant Functions and Logistic Regressions

Multivariate discriminant functions (DF1-DF5) and logistic regressions (LOGR1-LOGR5) were developed for different combinations of variables in direct and stepwise analyses (table 4). For all DFs and LOGRs the sectioning point is set to zero. Values greater than zero indicate a male individual and values less than zero a female. DF1 used all variables in a direct analysis and resulted in an overall 100% accuracy for the original and an overall 94% accuracy for the validation sample with 2.19% sex bias for the validation sample. DF2 employed the same variables in the stepwise analysis and resulted in selecting four variables (MAXH, MINB, DAFB and DAFL). Overall DF2 gave 100% accuracy for the original and 93.78% accuracy for the validation sample with the same sex bias. The DF3, DF4 and DF5 employed the Length (MAXL, LAL,DAFL), Height (MAXH,CFH,BH) and Breadth (MINB,MIDB, DAFB) variables and overall showed between 84.87% and 93.84% accuracy for the validation sample. The highest sex bias (-10.76%) was noted for DF3 for the validation sample.

Logistic regressions (LOGR1-LOGR5) were developed for all variables (direct and stepwise) and for Length, Height and Breadth variables resulting in 82.43% to 97.5% classification accuracy (see table 4). LOGR1 gave the highest classification accuracy (97.5%) and the lowest sex bias (-0.13%) for the validation sample. Although LOGR5 resulted in an overall 91.46% classification accuracy, it misclassifies females as compared to males with a sex bias of 17.07%.

When comparing the two methods, LOGRs gave the overall higher accuracies and lowest sex bias' in all cases for the original sample and in three of the five equations for the validation sample. Overall, for the validation sample, the formula with the highest accuracy rate is LOGR1 (table 4).

Discussion

Sex estimation is a fundamental step in creating the biological profile of heavily decomposed remains where identification is an issue. While the pelvis is considered the most accurate method for this task [1] often times it is fragmented or missing and other bones are used instead. The current study is focusing on the sexual dimorphism of the calcaneus, a resistant bone that is usually recovered intact in contemporary remains. The study population comes from Turkey, a country that lacks modern osteological collections due to religious prohibition [28]. Thus, CT scans of the calcaneus of living patients were used with the objective to create biological standards for sex estimation applicable to forensic cases in this area of the world. This is particularly relevant as currently 348 mass graves were discovered containing over 4000 individuals that require identification [27].

Sexual dimorphism of the calcaneus has been confirmed so far for several populations such as Italians [35,36], Greeks [12,16], South Africans [37,38], Japanese [21,39], Colombians [40], Koreans [41], and Northern Americans [22,23]. Discriminant functions were created and classification accuracy reached 96% in some occasions [36]. Table 5 illustrates the range of overall classification accuracies achieved for these studies compared to ours.

A common problem in comparing the results of these studies is the fact that measurements are not always named or described consistently; measurements with the same name may have different definitions and the same measurement may have more than one name. For example LAL is defined by Martin and Saller [33] as “the projected distance into the horizontal plane from the most posterior point of the tuber calcanei to the most anterior point of the posterior facet for the talus”. This measurement is adopted by some researchers [e.g. 39] while other studies use the same abbreviation LAL and define the measurement as “the linear distance between the most anterior point on the calcaneus and the most posterior point on the posterior articular facet” [e.g. 12, 26] which is clearly a different measurement. Another example is the variable MAXW in Bidmos and Asala [38] which is actually the same as the variable MIDB in Bidmos [26].

Our study followed the description of Martin and Saller [33] and Bidmos [26] to obtain nine measurements on the calcaneus. The data was analysed using two methods: DFA and LOGR. A second sample (N=80 individuals) was used to validate the functions produced by the statistical analysis. The results showed small differences in classification accuracy of the original and validation samples between the two methods. Yet, the LOGR consistently achieved lower sex bias' in classification accuracy compared to DFA. In our results, the MIDB is the best single variable regardless of which method of analysis we used. The MAXL also performed well, achieving up to 90% overall accuracy with very low sex bias (-0.5%) for the validation sample. This agrees with previous studies that found the best performing single variable to be the maximum length (MAXL) with accuracy ranging from 75.8% for modern southern Italians [36] to 83.5% for modern Greeks from Athens [12].

Multivariate functions achieved a great separation of the two sex groups reaching up to 97.5% classification accuracy for the validation sample. These results suggest that sexual dimorphism is very high for this bone in agreement with other studies that showed 94% to 96% accuracy rates [37,39]. The degree of sexual dimorphism is related to a number of factors such as genetics [42], diet [43-44] and occupational differentiation [13, 16, 45] that cannot be controlled in our sample. Nevertheless, a thorough discussion of possible explanations for the high degree of sexual dimorphism of the calcaneus in our sample is appropriate.

The bone mineral density of the calcaneus exhibits a high correlation with body weight, height, body fat and BMI as well as high calcium intake and physical activity [46]. Recent studies on the sexual dimorphism of the calcaneus and the metacarpal bones in Greeks suggested that increased sexual dimorphism in the capital Athens could be associated with less “urban” activities for females compared to a sample from Crete where it can be assumed that females were more engaged in “rural” activities [13,16]. A similar division of labour in the Athens sample could be the reason for the high sexual dimorphism in our study sample. Yet, no information is available on the occupation and physical activity of our subjects in order to confirm or reject this hypothesis.

Population differences constitute another factor affecting the results of this study compared to other studies. Northern Italians [36] show increased sexual dimorphism (up to 95.7%) in contrast to African Blacks for whom the overall classification accuracy was below 80% [38]. The Turkish sample is more homogenous compared to the sample from North America studied by DiMichelle and Sprandley [22] for instance, which includes individuals with different genetic composition. In fact, the aforementioned study used a mix sample of American Black, American White and Hispanic individuals and resulted in overall classification that barely reached 87%. In addition, different studies used samples of different chronology; thus, secular change may be another factor responsible for the differences in the expression of sexual dimorphism.

Last, high sexual dimorphism in our sample may be partially attributed to the “sampling effect” [16]. This means that the composition of the sample creates a positive bias towards higher classification accuracy. Yet, the sample size in our study is vastly large and equally distributed in the two sex groups, which gives high credibility to the results obtained here. In addition, the observer error in our study was low suggesting that the high accuracy cannot be attributed to measurement error. One could argue that measurements taken on virtual models, as in our case, may introduce an error in the study as they are generally considered less reliable compared to measurements on the dry bone. Recent validation studies on the cranium/long bones [47] and pelvis [48] suggest that the measurement error on virtual bones does not exceed 2mm, which implies high reliability. No validation study exists to date for virtual measurements of the calcaneus compared to measurements on the dry bone. We do not however anticipate the error to exceed the acceptable threshold of 2mm that was reported for other skeletal elements [47-48].

In conclusion, the calcaneus bone was found to be very sexually dimorphic in the Turkish population reaching an overall accuracy rate of 97.5% for the validation sample. This is actually the highest accuracy rate for sex estimation for this population as other studies on the crania [30, 32] and tibia [28] did not exceed 90%. In addition, the comparison between DFA and LOGR resulted in similar classification accuracy with lower sex bias in most of the cases when the LOGR was used. The fact remains that LOGR is adequate for non-parametric data but there is not enough evidence to support that the use of logistic regression is a more accurate classification method than discriminant function analysis. To increase the reliability of sex estimation based on the calcaneal measurements we recommend the use of both formulae (LOGR and DFA) depending on the preservation of the bone. It must be stressed that the formulae presented here are adequate for the Turkish population and should not be used in other circumstances without further testing.

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Conflict of interest

The authors have no conflict of interest

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Figure Legend:

Figure 1. Schematic drawing of calcaneal measurements: maximum length (MAXL), load arm length (LAL), dorsal articular facet length (DAFL), middle breadth (MIDB), minimum breadth (MINB), dorsal articular facet breadth (DAFB), maximum height (MAXH), body height (BH), and cuboidal facet height (CFH).

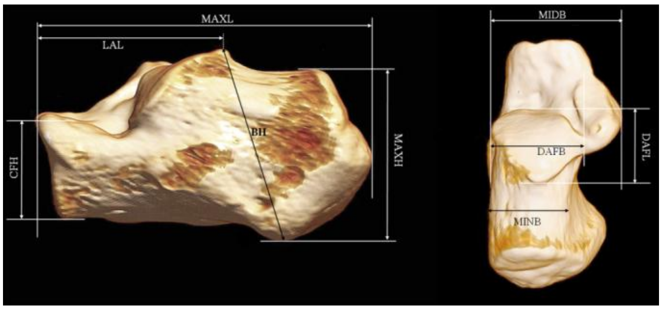

Table 1: Estimation of Intra- and Inter-observer error using TEM, *r*TEM and R.

	Intra-Observer Error (N=40)			Inter-Observer Error (N=40)		
	TEM	rTEM	R	TEM	rTEM	R
MAXL	0.84	1.04	0.98	1.22	1.50	0.96
MAXH	1.14	2.50	0.93	1.49	3.25	0.88
CFH	0.49	1.89	0.97	0.84	3.18	0.90
BH	0.72	1.73	0.97	0.90	2.16	0.95
MINB	0.33	1.40	0.97	0.45	1.88	0.94
DAFB	1.13	3.99	0.81	0.84	2.94	0.90
MIDB	0.84	2.05	0.92	0.84	2.02	0.92
LAL	0.49	1.04	0.98	0.75	1.58	0.96
DAFL	0.80	2.97	0.87	0.85	3.15	0.86

Table 2: Means, standard deviations and t-values for mean differences between males and females for all calcaneal variables.

	Males=179		Females=169		
	Mean	SD	Mean	SD	*t-value
MAXL	85.74	4.57	75.95	3.44	22.64
MAXH	49.39	2.96	42.37	1.99	26.09
CFH	28.43	1.73	24.05	1.37	26.26
BH	44.58	2.33	38.76	2.11	24.34
MINB	25.53	0.94	22.26	0.71	36.43
DAFB	30.77	1.09	26.09	1.07	40.58
MIDB	43.90	1.73	39.11	1.58	26.10
LAL	50.60	1.84	44.59	2.15	28.09
DAFL	28.77	1.25	24.99	1.29	27.72

*p<0.0001

Table 3: Univariate DFs and LOGRs, demarking points, classification accuracy and sex bias for the original and the validation sample.

		DP	Original sample				Validation sample			
			Males (N=179)	Females (N=169)	% sex bias	Overall (N=348)	Males (N=39)	Females (N=41)	% sex bias	Overall (N=80)
MAXL	DF	80.8	84.4	95.9	-11.5	89.9	89.7	90.2	-0.5	90.0
	LOGR	79.4	88.8	92.5	-3.7	90.5	89.7	85.4	4.3	87.6
MAXH	DF	45.9	89.4	98.8	-9.4	94.0	74.4	95.1	-20.7	84.8
	LOGR	44.8	91.0	97.0	-6.0	94.0	82.1	92.7	-10.6	87.4
CFH	DF	26.2	89.9	92.9	-3.0	91.4	66.7	92.7	-26.0	79.7
	LOGR	25.8	93.9	91.7	2.2	92.8	69.2	92.7	-23.5	81.0
BH	DF	41.7	85.4	92.3	-6.9	88.9	92.3	73.2	19.1	82.8
	LOGR	41.0	88.8	91.7	-2.9	90.2	94.9	70.7	24.2	82.8
MINB	DF	23.9	100.0	100.0	0.0	100.0	100.0	80.5	19.5	90.3
	LOGR	23.8	100.0	100.0	0.0	100.0	100.0	78.0	22.0	89.0
DAFB	DF	28.4	99.4	97.6	1.8	98.6	89.7	97.6	-7.9	93.7
	LOGR	28.5	99.4	98.2	1.2	98.8	89.7	95.1	-5.4	92.4
MIDB	DF	41.5	92.2	94.7	-2.5	93.4	84.6	92.7	-8.1	88.6
	LOGR	41.1	93.9	94.7	-0.8	94.3	89.7	87.8	1.9	88.8
LAL	DF	47.6	96.1	98.8	-2.7	97.4	76.9	87.8	-10.9	82.4
	LOGR	47.2	97.2	98.8	-1.6	98.0	76.9	85.4	-8.5	81.2

DAFL	DF	26.9	96.6	91.1	5.5	94.0	76.9	87.8	-10.9	82.4
	LOGR	26.8	95.0	91.7	3.3	93.4	76.9	87.8	-10.9	82.4

Table 4. Multivariate Univariate DFs and LOGRs, classification accuracy, and sex bias for the original and the validation sample.

		DF1	LOGR1	DF2	LOGR2	DF3	LOGR3	DF4
*Formulae	MAXL	-0.039	-1.093			0.083	0.024	
	MAXH	0.103	0.737	0.114				0.167
	CFH	0.098	0.682					0.364
	BH	0.042	0.630					0.167
	MINB	0.602	10.979	0.601				
	DAFB	0.411	4.442	0.426	169.584			
	MIDB	-0.013	0.735					
	LAL	0.091	1.149	0.077		0.23	1.845	
	DAFL	0.267	3.244	0.286	-58.75	0.457	1.304	
	Constant	-42.949	-547.88	-43.033	-6446.16	-29.958	-124.13	-24.14
Original sample	Males	100.00	100.00	100.00	100.00	98.30	97.80	95.50
	Females	100.00	100.00	100.00	100.00	97.60	98.20	97.60
	sex bias	0.00	0.00	0.00	0.00	0.70	-0.40	-2.10
	Overall	100.00	100.00	100.00	100.00	98.00	98.00	96.50
Validation sample	Males	94.87	97.44	94.87	89.74	79.49	79.49	87.18
	Females	92.68	97.56	92.68	100.00	90.24	85.37	97.56
	sex bias	2.19	-0.13	2.19	-10.26	-10.76	-5.88	-10.38
	Overall	93.78	97.50	93.78	94.87	84.87	82.43	92.37

* Sectioning points are set to zero in all cases.

Table 5. Comparison of classification accuracy between different populations

Population	M	F	Total N	% Accuracy	Publication
South Italians	40	40	80	76.25-85	Introna et al., 1997
Northern Italians	62	56	80	87.9-95.7	Gualdi-Russo, 2007
Athenians (Greece)	103	95	198	77.8-93.1	Peckmann et al., 2015
Cretans (Greece)	67	67	134	*80.3-85.3	Nathena et al. 2017
South Africans Whites	63	60	123	80.9-90.6	Bidmos et al., 2003
South Africans Blacks	58	58	116	63.8-79.3	Bidmos et al., 2004
Northern Americans	60	59	119	79-89	Steele et al., 1976
	184	136	320	80.1-86.7	DiMichelle and Sprandley, 2011
Colombians	84	50	134	67.4-89.5	Moore et al., 2016
Japanese	72	71	143	85-94	Sakaue et al., 2011
	108	84	192	59.5-84	Kawamoto et al., 2016
Koreans	50	54	104	65.4 -89.4	Kim et al. 2013
Turkish	179	169	348	88.9-100	this study

*Note: This study reported only classification accuracies over 80%